Candidate Tumor Suppressor and pVHL Partner Jade-1 Binds and Inhibits AKT in Renal Cell Carcinoma

Liling Zeng1, Ming Bai2, Amit K. Mittal1, Wassim El-Jouni3, Jing Zhou3, David M. Cohen4, Mina I. Zhou1, and Herbert T. Cohen1

Abstract
The von Hippel–Lindau (VHL) tumor suppressor pVHL is lost in the majority of clear-cell renal cell carcinomas (RCC). Activation of the PI3K/AKT/mTOR pathway is also common in RCC, with PTEN loss occurring in approximately 30% of the cases, but other mechanisms responsible for activating AKT at a wider level in this setting are undefined. Plant homeodomain protein Jade-1 (PHF17) is a candidate renal tumor suppressor stabilized by pVHL. Here, using kinase arrays, we identified phospho-AKT1 as an important target of Jade-1. Overexpressing or silencing Jade-1 in RCC cells increased or decreased levels of endogenous phospho-AKT/AKT1. Furthermore, reintroducing pVHL into RCC cells increased endogenous Jade-1 and suppressed endogenous levels of phospho-AKT, which colocalized with and bound to Jade-1. The N-terminus of Jade-1 bound both the catalytic domain and the C-terminal regulatory tail of AKT, suggesting a mechanism through which Jade-1 inhibited AKT kinase activity. Intriguingly, RCC precursor cells where Jade-1 was silenced exhibited an increased capacity for AKT-dependent anchorage-independent growth, in support of a tumor suppressor function for Jade-1 in RCC. In support of this concept, an in silico expression analysis suggested that reduced Jade-1 expression is a poor prognostic factor in clear-cell RCC that is associated with activation of an AKT1 target gene signature. Taken together, our results identify 2 mechanisms for Jade-1 fine control of AKT/AKT1 in RCC, through loss of pVHL, which decreases Jade-1 protein, or through attenuation in Jade-1 expression. These findings help explain the pathologic cooperativity in clear-cell RCC between PTEN inactivation and pVHL loss, which leads to decreased Jade-1 levels that superactivate AKT. In addition, they prompt further investigation of Jade-1 as a candidate biomarker and tumor suppressor in clear-cell RCC. Cancer Res; 73(17); 5371–80. ©2013 AACR.

Introduction
Renal cancer is a major clinical problem (1). In the United States, 65,150 new cases and 13,680 deaths were predicted from cancer of the kidney and renal pelvis for 2013 (2). More than 90% of kidney cancers are believed to originate from renal epithelial cells and are therefore referred to as renal cell carcinomas (RCC; ref. 3). The majority of cases of clear-cell RCC, the most common subtype of RCC, are characterized genetically by the loss, mutation, or silencing of the VHL gene (4, 5), making the von Hippel–Lindau (VHL) tumor suppressor pVHL– the major renal tumor suppressor in adults. However, the pathogenesis of renal cancer remains unresolved.

Serine/threonine kinase AKT is a key factor of perhaps the most frequently activated proliferation and survival pathway in cancer (6). Elevated AKT activity is also found in RCC and kidney cysts. Cystic lesions of patients with VHL show hyper-activated PI3K/AKT signaling (7). Increased phospho-AKT levels were found in about 50% of RCC tumor samples, and most commonly in the clear-cell subtype (8). Combined mutations of VHL and PTEN, a phosphatidylinositol (3–5)-trisphosphate phosphatase that negatively regulates the AKT/PKB signaling pathway, leads to kidney cysts in mice (7). PTEN-inactivating mutations (9, 10) or decreased PTEN expression have been identified in about 30% of clear-cell RCCs (11–13). Hyperactivation of AKT due to conditional knockout of neofi bromatosis type II (Nf2) in mouse renal proximal tubules leads to invasive RCC (14). Human renal cancer cell lines also show constitutive activation of AKT, and PI3K/AKT inhibitor treatment induces apoptosis and inhibits cell growth in vitro and in xenografts (15). Thus, AKT is activated in clear-cell RCC, but the mechanism has not always been apparent.

Jade-1, a short-lived protein most highly expressed in renal proximal tubules, was identified as a novel strong binding partner of pVHL (16). Wild-type pVHL stabilizes Jade-1, whereas renal cancer-causing forms cannot (17). Jade-1 is a candidate renal tumor suppressor and promotes apoptosis (18). Jade-1 functions as a ubiquitin ligase to inhibit canonical Wnt signaling (19) and as a transcription factor associated with...
Materials and Methods

Constructs

For pSUPERJade-1sh (for constant Jade-1 knockdown) or pSUPERIOR.neo/jade-1sh (for inducible Jade-1 knockdown) constructs, siRNA duplex DNA oligomers (sequences can be obtained from the authors) were ligated into pSUPER or pSUPERIOR.neo vector (OligoEngine) using BglII and HindIII sites. pJade-1sh1, pJade-1sh2 and nonsilencing vector were from Open Biosystems/Thermo Scientific. Flag-Jade-1 plasmid, myc-Jade-1 full length and deletion mutants del1, del2, del3, del4, J-N, J-La, and J-Sm have been described in (16, 19, 21). Adenovirus full length and deletion mutants del1, del2, del3, del4, J-N, J-La, and J-Sm have been described in (16, 19, 21). Adenovirus expressing myrAKT, dnAKT, or control β-gal was described in (26). pCMV5-based HA-AKT constructs were generated by site-directed mutations: dd (T308D/S473D), aa (T308A/S473A), and ki (K179M). To make GST-tagged AKT-P/L, AKT-cat, and AKT-349, or 350 were PCR-cloned into vector pGEX-6P-1 using BamHI and SalI sites.

Antibodies

Anti-Jade-1 rabbit polyclonal serum was described in (16). AKT, phospho-AKT S473, phospho-AKT T308, AKT1, AKT2, phospho-AKT substrate (RXRXXS/T’’), GSK3β, phospho-GSK3β Ser9, Erk1/2, pErk1/2, HA, and myc antibodies were from Cell Signaling Technology. pVHL and p21 antibodies were from BD Pharmingen. pVHL and p21 antibodies were from Cell Signaling Technology. pVHL and p21 antibodies were from BD Pharmingen.

Results

Jade-1 inhibits phospho-AKT/AKT1 in renal cell lines

Because Jade-1 orthologs participate in signal transduction, we used a phospho-MAPK array kit to look for signaling pathways in which Jade-1 is involved. In tetracycline-inducible Jade-1 knockdown HEK293 cells, tetracycline treatment induced Jade-1 short hairpin RNA expression such that the endogenous level of Jade-1 was knocked down to 40% compared with a control without tetracycline (Fig. 1A, left). With Jade-1 knockdown, the level of endogenous phospho-AKT1 increased by 2.3-fold (Fig. 1B, top). Conversely, stable over-expression of Jade-1 (Fig. 1A, right) decreased the level of endogenous phospho-AKT1 to 40% compared with empty vector control (Fig. 1B, bottom). Phospho-AKT2 was also regulated similarly by Jade-1 but to a lesser degree (Fig. 1B), whereas phospho-p38α (T180/Y182) and phospho-p38γ (T183/Y185) were not regulated by Jade-1 (data not shown).

Observations with the phospho-MAPK array were confirmed in transient transfection experiments. Knockdown of Jade-1 with pSUPERJade-1sh enhanced endogenous AKT1 S473 and T308 phosphorylation by 2-fold (Fig. 1C). Endogenous phospho-AKT2 was only slightly increased with Jade-1 knockdown, and endogenous pErk1/2 (Erk1 T202/Y204, Erk2 T185/ Y187) was not affected by Jade-1 knockdown. Overexpression of Jade-1 decreased the endogenous phospho-AKT1 level to 30% of control (Fig. 1D, left). Reintroduction of Jade-1 into the Jade-1 knockdown cells restored the endogenous phospho-AKT1 levels (Fig. 1D, right). Thus, Jade-1 specifically regulates phospho-AKT1 without affecting the total endogenous AKT1 level.
Figure 1. Jade-1 binds and inhibits AKT in renal cell carcinoma.

To increase efficiency of Jade-1 knockdown in a model more relevant to renal cancer, we used lentivirus-mediated knockdown in HK-2 cells, a differentiated human proximal tubule cell line (28) that represents a model of renal cancer precursor cells. In Jade-1-silenced Jade-1sh1 and Jade-1sh2 HK-2 cell lines, levels of endogenous phospho-AKT1 S473 and T308 increased about 2-fold, and total AKT1 levels remained unchanged (Fig. 2A). Strikingly, Jade-1 silencing increased total endogenous phospho-AKT levels by 2–3-fold (Fig. 2A). Regulation of phospho-AKT by Jade-1 was inhibited by PI3K inhibitors wortmannin and LY294002. In contrast, endogenous pErk1/2 was not affected by Jade-1 silencing in the absence or presence of LY294002. In addition, reintroduction of silencing-resistant pFlag-Jade-1 into the Jade-1sh2 cells decreased the levels of phospho-AKT to 50% (Fig. 2B).

We also examined phospho-AKT during IGF-1 treatment. Phospho-AKT in nonsilencing cells was fully activated within 5 minutes. In comparison, phospho-AKT levels in Jade-1sh1 cells increased gradually and reached a 2-fold maximum level (compared with nonsilencing cells) in 30 minutes (Fig. 2C).

We examined the effects of Jade-1 and pVHL on phospho-AKT in 786-O renal cancer cells. Stable overexpression of Jade-1 in 786-O renal cancer cells inhibited endogenous phospho-AKT (Fig. 2D). Pooled 786-O stable cell lines expressing either empty vector or HA-tagged pVHL were obtained low phospho-AKT levels (Fig. 2E). Silencing of Jade-1 in the 786-O VHL cells maintained low phospho-AKT levels (Fig. 2E), suggesting that the effect of pVHL on AKT is
Jade-1 dependent. Thus, the regulation of phospho-AKT by Jade-1 is valid, specific, and relevant to renal cancer.

**Jade-1 interacts with AKT**

Endogenous Jade-1 was coimmunoprecipitated with endogenous AKT and *vice versa* (Fig. 3A). Confocal microscopy revealed that Jade-1 partly colocalized with AKT. This colocalization was observed inside the cell, in the cytoplasm and nucleus, when cells were at low confluence (Fig. 3B), or more consistently near the cell membrane when cells were at confluence (Fig. 3C).

To map the AKT-binding domain of Jade-1, HA-AKT1 was coexpressed with deletion/truncation mutants of myc-Jade-1 (Fig. 4A). AKT1 only binds full-length, del3 and del4, but not del1 or del2 (Fig. 4B, left). AKT1 only binds the Jade-1 N terminus (although with a reduced affinity compared with full-length Jade-1), but not the double-PHD domain J-La or the interferon region J-Sm (Fig. 4B, right). Collectively, the N terminus of Jade-1 seems to be necessary for binding AKT1. Kinase inactive (ki) AKT shows much stronger binding with Jade-1 than other forms of AKT (wt, dd, aa; Fig. 4C), implying that Jade-1 makes important and potentially functional contact with the AKT catalytic domain. To determine the Jade-1-binding domain of AKT, myc-Jade-1 was incubated with GST, GST-AKT-PH/Linker, GST-cat, or GST-tail (Fig. 4D). Only the AKT catalytic domain and C-terminal tail can bind myc-Jade-1 (Fig. 4D). Thus, Jade-1 likely inhibits AKT kinase activity by binding both the AKT kinase domain and regulatory tail.

We also tested whether AKT could be a ubiquitylation target of Jade-1, which is an E3 ubiquitin ligase for the oncoprotein β-catenin (19). Ubiquitylated AKT was not detectable when
Jade-1 regulates AKT by binding AKT and inhibiting phospho-AKT, but not through AKT ubiquitylation.

Jade-1 silencing affects AKT kinase activity and downstream targets

Next, we examined the molecular effects of elevated levels of phospho-AKT in Jade-1-silenced HK-2 cells. AKT in vitro kinase assays confirmed elevated AKT kinase activity in Jade-1-silenced cells on purified GSK3β (Fig. 5A). phospho-AKT substrate (RXRXXS/F) antibody was then used to profile phospho-AKT substrates in the HK-2 cell lines. Jade-1 silencing enhanced several endogenous phospho-substrates of AKT without changing global AKT substrate phosphorylation (Fig. 5B), showing selective modulation of AKT substrates in these renal cancer precursor cell lines. As one example, Jade-1 silencing increased levels of endogenous phospho-GSK3β S9 (Fig. 5C). Levels of endogenous p21, a downstream target (29) negatively regulated by phospho-AKT (30, 31), decreased to 40–50% in Jade-1-silenced cells (Fig. 5D). This effect was attenuated with LY294002. Thus, Jade-1 affects AKT activity and downstream targets.

Jade-1 silencing promotes cell proliferation in renal cancer precursor cells

Cell-growth assays were conducted. Both Jade-1–silenced cell lines grew faster than the nonsilencing control cells (Fig. 6A). In addition, BrdUrd incorporation assays revealed that silencing of Jade-1 significantly increased cell proliferation 25% to 35% (Fig. 6B). Thus, Jade-1 is involved in cell-cycle progression and cell proliferation in renal cancer precursor cells.

Jade-1 silencing promotes anchorage-independent growth in renal cancer precursor cells

The PI3K/AKT pathway plays an important role in anchorage-independent growth, a key feature of transformed epithelia. Soft agar assays were conducted on HK-2 cells, which normally do not grow in soft agar (28). Intriguingly, Jade-1–silenced HK-2 cells acquired the capacity for anchorage-independent survival and growth (Fig. 6C). This capacity was inhibited by reintroduction of Jade-1 (Fig. 6D) or LY294002 (Fig. 6E); moreover, it was suppressed by dominant-negative AKT and enhanced by constitutively active myrAKT (Fig. 6F). myrAKT alone was barely able to transform the nonsilencing control cells (Fig. 6F, top right). Therefore, Jade-1–silenced renal cancer precursor cells exhibit phospho-AKT–dependent transformation.

Jade-1 message levels are low in clear-cell RCC, and low Jade-1 is associated with worse patient features and activation of an AKT1 target-gene signature

We analyzed The Cancer Genome Atlas database for differential gene expression (P = 0.001, false-discovery rate < 0.001) between clear-cell RCCs (n = 65) and corresponding normal kidney tissue (n = 65) from the same patient. Jade-1 message, but not AKT1 message, was significantly underexpressed in clear-cell RCC samples, with a ratio of 0.74. Furthermore, in all clear-cell RCCs in the database, low levels of Jade-1 message (n = 232) were significantly associated with advanced tumor stage, high pathologic grade and more patient deaths, in comparison with clear-cell RCCs with high Jade-1 expression (n = 232; Table 1). In contrast, AKT1 message levels were not

HEK293 cells were transfected with Jade-1 and/or treated with proteasome inhibitor MG132 (data not shown). Our data also showed that Jade-1 did not affect the total AKT level. Thus, Jade-1 regulates AKT by binding AKT and inhibiting phospho-AKT, but not through AKT ubiquitylation.
significantly associated with these clinical characteristics (data not shown). Most likely, AKT1 transcript levels are not strongly correlated with active AKT1 protein levels. Importantly, the low Jade-1–expressing clear-cell RCC samples exhibited overexpression of genes positively regulated by AKT1 and underexpression of genes negatively regulated by AKT1 (Supplementary Table S1), consistent with the activation of an AKT1 target-gene signature (32, 33) under low Jade-1 conditions.

Discussion

Although most cases of clear-cell RCCs involve inactivation of the pVHL tumor suppressor (1, 3), AKT is abnormally activated in about half of cases (8), sometimes due to PTEN mutation (9, 10) or more often from decreased PTEN expression (11–13). Other mechanisms for activation of AKT in RCC have been unknown, but cooperativity between the PTEN and pVHL pathways has been described (7). Jade-1 is a highly regulated, antiproliferative, and proapoptotic molecule enriched in kidney epithelial cells. Loss of Jade-1 stabilization by pVHL correlates with renal cancer risk (17). Here, we provide further evidence for the renal tumor suppressor role of Jade-1 as an inhibitor of phospho-AKT. Overexpression of Jade-1 decreased and silencing of Jade-1 increased the level of endogenous phospho-AKT/AKT1 in renal cell lines. By increasing Jade-1 abundance, reintroduction of pVHL into renal cell lines (10% of input) showed comparable expression of HA-AKT or myc-Jade-1 truncations across samples. Jade-1 shows increased binding to a kinase inactive AKT mutant. Myc-Jade-1 was transiently cotransfected with different forms of HA-AKT, wild-type (wt); T308D/S473D (dd); T308A/S473A (aa); or K179M (ki). Jade-1 binds to the AKT kinase domain and the regulatory C terminus. Cell lysates with overexpressed myc-Jade-1 were incubated with bacterially expressed, purified GST fusion protein GST-AKT-P/L, GST-AKT-cat, GST-AKT-tail, or GST alone.

Figure 4. The N-terminus of Jade-1 binds both the catalytic domain and the C-terminal regulatory region of AKT. A, schematics of myc-tagged Jade-1 truncation constructs and GST-tagged AKT domain constructs. B, AKT binds the N terminus of Jade-1. HA-AKT was transiently cotransfected in HEK293T cells with myc-Jade-1 truncations (left, full length, del1, del2, del3; and right, full length, J-N, J-La, J-Sm). Cell lysates were immunoprecipitated with anti-myc antibody and then immunoblotted with anti-HA antibody, and vice versa. Whole-cell lysates (10% of input) showed comparable expression of HA-AKT or myc-Jade-1 truncations across samples. C, Jade-1 shows increased binding to a kinase inactive AKT mutant. Myc-Jade-1 was transiently cotransfected with different forms of HA-AKT, wild-type (wt); T308D/S473D (dd); T308A/S473A (aa); or K179M (ki). Jade-1 binds to the AKT kinase domain and the regulatory C terminus. Cell lysates with overexpressed myc-Jade-1 were incubated with bacterially expressed, purified GST fusion protein GST-AKT-P/L, GST-AKT-cat, GST-AKT-tail, or GST alone.
suppressor function of Jade-1 is due in part to inhibition of AKT activity. We have also found that, in comparison with normal kidney, Jade-1 message is significantly reduced in clear-cell RCC, which is associated with poor prognosis and activation of an AKT1 target-gene sequence, consistent with Jade-1 serving as a key regulator of the PI3K/AKT pathway.

Jade-1 abundance is low to undetectable in renal cancer cell lines but is high in differentiated renal proximal tubular cells (18). Our results show that when Jade-1 levels fall, hyperactivated AKT in renal cancer precursor cells could facilitate their survival and proliferation, and presumably make them less sensitive to apoptotic signals. The surviving cells might reprogram energy metabolism, for example, through GSK3β (34). These cells could outgrow other normal cells by manipulating cell-cycle-related proteins such as p21 and p27, to get a proliferation advantage. In addition, hyperactivated AKT in renal cancer precursor cells favors anchorage-independent growth, which is critical for epithelial–mesenchymal transition, a process through which epithelial cells acquire deregulated cell polarity, reduced intercellular adhesion, and increased invasion/metastasis. Thus, increased AKT activation could contribute to renal tumorigenesis in multiple ways.

AKT signaling is a ubiquitous eukaryotic pathway, and complex mechanisms exist for fine control of AKT. AKT-interacting proteins can modify AKT kinase activity or target specificity. For example, by binding the AKT PH domain, oncoprotein Tcl1 activates AKT and promotes its translocation to the nucleus (35). TRB3 binds the activation loop of the AKT kinase domain and inhibits AKT phosphorylation (36). CTMP binds specifically to the C-terminal regulatory region of AKT1 at the plasma membrane and negatively regulates AKT1 activation (37). The mechanism of Jade-1 inhibition of AKT may be unique because Jade-1 binds both the AKT kinase and regulatory domains.

AKT undergoes a substantial conformational change upon phosphorylation of T308 and S473 (38). Following this, ATP binding K179 in the catalytic cleft of AKT induces interactions between the phosphorylated sites and other residues such as H194 and R273 in the cleft, creating a "phosphatase shielding cage" (39, 40). ATP hydrolysis and substrate phosphorylation "uncage" AKT phosphorylated sites, leading to dephosphorylation and inactivation of AKT. An inherited AKT2 R274H mutation blocking "cage" formation leads to AKT2 kinase inhibition and results in severe diabetes mellitus in humans (41). The K179M mutation abolishes the ATP-binding site, so AKT K179M adapts an "uncaged" conformation. Our data show that Jade-1 binds AKT K179M with much higher affinity than the other forms of AKT tested, suggesting that overexpressed Jade-1, through its strong retention of "uncaged" AKT, could facilitate phosphatase accessibility, leading to lower steady-state levels of endogenous phospho-AKT. This assumption is strengthened by our data: (i) Jade-1 binds both the AKT catalytic domain and C-terminal regulatory tail, implying Jade-1 may affect the AKT phosphorylation sites, and (ii) when cells are at confluence, colocalization of Jade-1 and AKT is highest near the cell membrane, where AKT phosphorylation occurs. A model of pVHL and Jade-1 control of phospho-AKT is presented (Fig. 6G). Thus, as a tightly regulated short-lived protein, Jade-1 can provide fine control of AKT phosphorylation and thereby AKT activity.

Figure 5. AKT activity and levels of endogenous phospho-substrates of AKT are increased in Jade-1-silenced HK-2 cells. A, endogenous AKT was immunoprecipitated from cellular extracts of HK-2 stable cell lines and then incubated with purified GSK3β substrate. The amount of phosphorylated GSK3β S9 was assessed by immunoblotting. B, cell lysates from HK-2 cell lines were probed with a phospho-AKT substrate antibody. C, levels of endogenous phospho-GSK3β S9, a known substrate of phospho-AKT, were assessed by immunoblot analysis. D, levels of endogenous p21 in the HK-2 cell lines were assessed with or without LY294002.
During AKT activation, increased Jade-1 preferentially binds “uncaged” AKT, shifting the balance toward this form of phospho-AKT that is more susceptible to dephosphorylation and inactivation. Short-lived protein Jade-1 is stabilized by pVHL.

Loss of PTEN plays an important role in RCC, but additional biomarkers are needed. The familial PTEN hamartoma and tumor syndrome results in a 30-fold increased risk of RCC (42). PTEN gene defects have been found in about 30% of RCCs (9, 10, 13, 43), which should increase phospho-AKT. A relationship between decreased PTEN expression and increased phospho-AKT has been identified in clear-cell, but not in papillary or chromophobe RCC (44). In an analysis of seven renal cancer cell lines, four VHL null and three VHL intact, all had constitutive phosphorylation of AKT (15), so there are likely to be more pathways to AKT activation in RCC than just PTEN or pVHL loss such as decreased Jade-1, which we have observed in all RCC cell lines tested (18). Loss of PTEN may be a poor prognostic finding (11, 12), but PTEN, HIF-1α, CA9, or pVHL status have not proved to be useful predictive biomarkers for therapy with mTOR inhibitors (13, 43). Instead, phospho-S6

**Figure 6.** Silencing of Jade-1 increases cell proliferation and promotes AKT-dependent anchorage-independent growth. A, cell numbers were counted with a hemocytometer for seven consecutive days. Error bars indicate SD. B, growing cells were labeled with BrdUrd solution (10 μmol/L, 1.5 hours). Incorporated BrdUrd was detected with anti-BrdUrd-POD solution (Roche) and visualized with 3, 3′-diaminobenzidine substrate (Vector Laboratories). Error bars indicate SD. C, Jade-1-silenced HK-2 cell lines formed colonies in soft agarose. HK-2 cell lines were grown in an agarose suspension. Formation of colonies was monitored microscopically. D, reintroduction of Jade-1 into Jade-1sh2 cells inhibited anchorage-independent growth. E, LY294002 inhibited anchorage-independent growth of Jade-1sh2 cells. F, anchorage-independent growth of Jade-1-silenced HK-2 cells is AKT-dependent. HK-2 stable cell lines were infected with adenovirus expressing either β-galactosidase (β-gal) control, dominant-negative AKT (dnAKT), or constitutively active AKT (myr-AKT) before they were suspended in soft agarose. Error bars indicate SD. G, a model of AKT inhibition by Jade-1/pVHL. Starting on the bottom left, serine/threonine kinase AKT is activated by phosphorylation of a substrate protein and ATP hydrolysis, AKT adopts an “uncaged” conformation that is more susceptible to dephosphorylation and inactivation. Short-lived protein Jade-1 is stabilized by pVHL. During AKT activation, increased Jade-1 preferentially binds “uncaged” AKT, shifting the balance toward this form of phospho-AKT that is more susceptible to inactivation by phosphatases. Conversely, in renal cancer, loss of Jade-1/pVHL would shift the balance toward the “caged” form of AKT, resulting in higher phospho-AKT levels and increased AKT kinase activity.
of the short-lived protein Jade-1 in renal cancer precursor
pVHL
VHL disease and renal cancer. This work indicates that
are important for phenotypes other than angiogenesis in
proteasomal degradation (45); however, non-HIF pathways
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Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer

Table 1. Patient clinical characteristics based on Jade-1 gene expression

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Abbreviation: ns, not significant.

ribosomal protein and perhaps phospho-AKT were predictive markers for response to temsirolimus in RCC (13). Moreover, only patients with high levels of phospho-S6 or phospho-AKT responded to temsirolimus (13). Low levels of jade-1 may prove to be a predictive biomarker for mTOR inhibitor therapy in RCC, as well as a prognostic biomarker.

pVHL is best understood as targeting the hypoxia-inducible factor α transcription factors for ubiquitination and proteasomal degradation (45); however, non-HIF pathways are important for phenotypes other than angiogenesis in VHL disease and renal cancer. This work indicates that pVHL fine tunes AKT kinase activity through stabilization of the short-lived protein Jade-1 in renal cancer precursor cells. In addition to Jade-1 regulation (16), pVHL can regulate NF-kB activity and tumorigenesis by serving as an adaptor to promote the inhibitory phosphorylation of the NF-kB agonist Card9 by kinase CK2 (46). pVHL has also been shown to regulate microtubules and primary cilium formation in kidney cells (47), endocytosis of fibroblast growth factor receptor 1 (48) and assembly of ECM (49). The regulation of Jade-1 protein and hence AKT by pVHL thus sheds new light on the molecular events in clear-cell RCC subsequent to VHL loss. Furthermore, Jade-1 expression seems to be dysregulated at the message level as well in clear-cell RCC through an unidentified mechanism. Candidate renal tumor suppressor Jade-1 is therefore a key regulator of multiple cell signaling pathways and normally prevents renal epithelial cell proliferation and transformation.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: L. Zeng, D.M. Cohen, M.I. Zhou, H.T. Cohen
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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): L. Zeng, M. Bai, A.K. Mittal, D.M. Cohen, M.I. Zhou, H.T. Cohen
Writing, review, and/or revision of the manuscript: L. Zeng, M. Bai, A.K. Mittal, J. Zhou, H.T. Cohen
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J. Zhou, H.T. Cohen
Study supervision: J. Zhou, M.I. Zhou, H.T. Cohen

Acknowledgments
The authors thank Z. Xiao and K. Walsh for generously providing reagents; D. Faller, T. Hsu, and Z. Luo for helpful discussions; R. Simon and colleagues for developing BIB-array tools (National Cancer Institute); and The Cancer Genome Atlas research network for producing and maintaining microarray databases.

Grant Support
This work was supported by NIH grants R01 CA079830 and R01 DK067569 (H.T. Cohen) and NIH grants R37 DK51050 and R01 DK03357 and March of Dimes grant 1-FY12-527 (J. Zhou).

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Received January 8, 2013; revised May 2, 2013; accepted June 1, 2013; published OnlineFirst July 1, 2013.

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